Minireview

Risks associated with vaccinia virus in the laboratory☆

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A B S T R A C T

Vaccinia virus (VACV) is used commonly in research laboratories. Non-highly attenuated strains of VACV are potentially pathogenic in humans, and VACV vaccination and biosafety level 2 facilities and protocols are currently recommended for vaccinated laboratory workers in the United States who handle non-highly attenuated strains of the virus. Despite this, laboratory-related VACV exposures continue to occur and a number of recent instances of VACV infection in non-vaccinated laboratory workers have been documented. We provide a discussion of the usage and risks associated with VACV in laboratory research.

Background

The practice of immunizing with orthopoxviruses dates back over 200 years, in which the concept of vaccination was introduced by Edward Jenner, to protect against variola virus infection, the causative agent of smallpox. While it is not entirely clear what orthopoxvirus was first used for vaccination practices, vaccinia virus (VACV) was eventually (if not initially) used as the viral agent for smallpox vaccination, and its successful usage was largely responsible for the global eradication of smallpox. Similarly, a natural reservoir for VACV has yet to be identified and the origins of the virus are not currently established (Baxby, 1977); explanations for the origin of VACV include (i) the possibility that this was a zoonotic virus that has since gone extinct in nature, (ii) that VACV was artificially derived from another orthopoxvirus through serial passage during vaccine production, and (iii) that VACV may be a recombinant between variola and the original species of orthopoxvirus that was used for vaccination (Binnis and Smith, 1992; Shchelkunov et al., 2005). Genetically, VACV is closely related to horsepoxvirus, however, the presence of gene disruptions in the horsepox genome, that are intact in the VACV genome, does not support the origin of VACV from horsepox (Tulman et al., 2006).

Vaccination with VACV provides long-lasting protection against a variety of orthopoxviruses, including variola and monkeypox viruses (Fine et al., 1988; Jezek et al., 1987, 1988). Among human vaccines, VACV is unique in that it is delivered as a live, non-attenuated virus, by puncture of the skin overlying the deltoid with a bifurcated needle. Because of this, adverse events following vaccination are known to occur, particularly among those with high risk conditions, such as individuals with eczema, atopic dermatitis, or other skin conditions, individuals who are immunocompromised or pregnant, and those less than 1 year of age (Casey et al., 2006; Cono et al., 2003; Wharton et al., 2003). Secondary transmissions to close contacts of vaccinees can also occur. Out of 37,901 volunteers vaccinated under the US Department of Health and Human Services preparedness program, 722 nonserious adverse events and 100 serious events, including 85 hospitalizations, were reported (Casey et al., 2005). Similarly, in a study of laboratory workers receiving VACV vaccine, a wide variety of post-vaccination symptoms were identified, although most common symptoms tended to be relatively mild (Baggs et al., 2005). In August 2007, the Food and Drug Administration (FDA) licensed the new-generation ACAM2000 vaccine, which is now used in place of the Dryvax vaccine, in the United States (CDC, 2008) and clinical trials suggest similar safety, as well as efficacy (Greenberg and Kennedy, 2008).

VACV in laboratory research

VACV is commonly used in modern molecular biology research. Fig. 1 shows cumulative yearly numbers of publications (from National Library of Medicine's PubMed database) with ‘Vaccinia’ in the title or abstract. While numbers of publications have increased slightly over the past decade, it is apparent that VACV usage has long been well-established in laboratory research. Consistent with this observation, VACV is used in the laboratory for a wide variety of purposes. As an example, we classified all abstracts, with ‘Vaccinia’ in the title or
A VACV strain is considered non-highly attenuated if the virus maintains the capacity to replicate productively in mammalian cells. Numerous non-highly attenuated strains of VACV currently exist. Of central importance to current laboratory research is the Western Reserve (WR) strain of VACV which was selected by serial intracerebral passage in mice, for neurotropic potential. Other strains used in the laboratory commonly are related to the New York City Board of Health (NYCBH) strain, from which the Dryvax and ACAM2000 vaccine strains, as well as VACV WR, were derived (ATCC, 2008; Wokatsch, 1972). In contrast, attenuated strains of VACV do not have the capacity to replicate in mammal cells. Of note is the modified vaccinia Ankara (MVA) strain, which is able to infect and result in protein expression, but not replicate, in mammalian cells (Sutter and Moss, 1992), and may serve as a possible alternative in the future to the currently licensed VACV vaccine (Phelps et al., 2007).

**Laboratory safety and VACV infections**

Because non-highly attenuated strains of VACV are pathogenic in humans and handling virus in the laboratory presents a possible risk of infection, safety guidelines have been developed. As a vaccine, VACV has been shown to be highly efficacious in generating protective immune responses against both smallpox and monkeypox, and furthermore, studies indicate robust VACV-specific immune responses in humans following vaccination (Amanna et al., 2006). Therefore, pre-exposure vaccination with VACV is a primary intervention that can prevent or minimize the effects of accidental exposure in the laboratory. Consistent with this observation, the Advisory Committee on Immunization Practices (ACIP) recommends VACV vaccination for laboratory workers in the United States who handle cultures or animals contaminated or infected with non-highly attenuated VACV strains, at least every 10 years (Rotz et al., 2001).

In addition to vaccination, the usage of at least Biosafety Level 2 practices and facilities are currently recommended for the manipulation of viruses or animals infected with non-highly attenuated VACV strains (2007). This includes the usage of proper personal protective equipment, including gown, gloves, and eyewear protection when procedures have the risk of splash, proper laboratory facilities and safety equipment, proper decontamination of infectious material, and proper animal facilities (2007).

Despite the availability of vaccination and worker adherence to safety procedures, laboratory-acquired VACV infections do occur and...
have been well documented in the literature. Inadvertent exposures have occurred through needlestick accidents or eye splash (2008; Jones et al., 1986; Lewis et al., 2006; Loeb et al., 2003; Mempel et al., 2003; Moussatche et al., 2003; Openshaw et al., 1991; Wlodaver et al., 2004). Laboratory-acquired VACV infections have commonly involved recombinant viruses, which express foreign proteins (2008; Jones et al., 1986; Lewis et al., 2006; Mempel et al., 2003; Openshaw et al., 1991) produced from non-highly attenuated strains, such as WR (2008; Jones et al., 1986; Lewis et al., 2006; Mempel et al., 2003; Moussatche et al., 2003; Openshaw et al., 1991).

There exists no formal surveillance system in place, within the United States, for instances of laboratory-related orthopoxvirus exposures or infections. However, the Poxvirus Team at the Centers for Disease Control and Prevention (CDC) has been contacted on a number of occasions in recent years regarding instances of laboratory-related orthopoxvirus exposures (Table 1). The majority of these instances have involved VACV (typically WR) harboring a foreign gene in the TK locus. Exposure to VACV most commonly occurred through accidental needlesticks or through eye splash accidents. In 6 recent instances, exposure resulted in VACV infection, and 4 of 6 infections resulted in subsequent hospitalization. Infections commonly involved fever and large focal areas of painful induration, erythema and severe swelling around the inoculation site. Hospitalizations occurred to assess the need for surgical or medical intervention. Five of the 6 cases involved infection of the hand or digits, and the remaining individual had ocular and facial involvement. None of the six infected laboratory workers had met the ACIP vaccination recommendation for working with non-highly attenuated VACV in the laboratory.

Discussion

VACV is commonly used in laboratory research and the occurrence of laboratory-related VACV infections has been documented previously (2008; Jones et al., 1986; Lewis et al., 2006; Loeb et al., 2003; Mempel et al., 2003; Moussatche et al., 2003; Openshaw et al., 1991; Wlodaver et al., 2004). In recent years, we have received a number of reports of laboratory-related VACV exposures and infections. Additionally, laboratory-acquired VACV infections commonly have been the result of TK-minus strains of virus. Because studies in mice have suggested that inactivation of the VACV TK locus results in decreased virulence (Buller et al., 1985; Lee et al., 1992), it may be perceived there is not a risk of infection associated with handling a TK-minus VACV strain; however, it is apparent that TK-minus strains of VACV do maintain pathogenic potential in humans.

Because we currently do not have an estimate on the number of laboratories, or researchers who are potentially exposed to non-highly attenuated VACV strains, or the actual number laboratory-associated VACV infections that occur, it is not possible to accurately assess the overall risk of VACV infection, associated with laboratory work. However, the observation that a large number of exposures, and all recent infections, involve individuals who did not follow the ACIP recommendations for vaccination warrants consideration for a number of reasons. It is apparent that many researchers are hesitant to be vaccinated, because it is perceived that the risk of adverse events associated with vaccination is higher than the risk of working with VACV. While the risks associated with vaccination are thoroughly described to those considering vaccination, the benefits of receiving vaccination are often overlooked. There are a number of reasons that researchers should consider vaccination:

1. Vaccination involves controlled delivery of the virus to the skin overlying the deltoid. This is a region of the body that can easily tolerate swelling without compromising function or causing significant pain. Accidental infection on other parts of the body (e.g., hand or digit) can result in severe pain and swelling, and possible long-term sequelae. Furthermore, many would consider the cosmetic effects of a vaccination scar on the deltoid to be less objectionable than scarring on another part of the body, such as the hand, eye, or face, which are locations commonly associated with accidental infection.

2. Vaccination involves inoculation with a controlled dosage of a well-characterized virus strain. By contrast, laboratory-related exposures can result in the delivery of a high titer of virus, as well as delivery through an atypical route, such as deep injection or ocular inoculation. Furthermore, laboratory studies commonly involve recombinant VACV strains, which have the potential to result in altered viral virulence or artificially modulated immune response to the virus.

3. Adverse events associated with vaccination are generally mild and severe adverse events are rare. For instance, 0.22% of vaccinations administered under the US Department of Health and Human Services preparedness program resulted in hospitalization (Casey et al., 2005). By contrast, 4 of the 6 (66%) recent laboratory-acquired VACV infections reported to CDC resulted in hospitalization.

4. Although the risk of exposure can be minimized by handling the virus under proper laboratory conditions and using proper techniques (for instance, the usage of eye protection can prevent ocular exposure), it is often not possible to completely eliminate the risk of accidental exposure to VACV. Personal protective equipment cannot provide complete protection from needlestick accidents, which account for a large proportion of VACV exposures.

### Table 1
Laboratory-related orthopoxvirus exposures reported to CDC, 2005–2008

<table>
<thead>
<tr>
<th>Year</th>
<th>State</th>
<th>Virus (strain, if known)</th>
<th>Met ACIP vaccination?</th>
<th>Nature of accident</th>
<th>Result in infection?</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td>CA</td>
<td>Vaccinia</td>
<td>No</td>
<td>Eye splash</td>
<td>No</td>
</tr>
<tr>
<td>2005</td>
<td>FL</td>
<td>Vaccinia (rabbitpox)</td>
<td>Yes</td>
<td>Eye splash</td>
<td>No</td>
</tr>
<tr>
<td>2005</td>
<td>CT</td>
<td>Vaccinia (recombinant WR)</td>
<td>No</td>
<td>Needlestick</td>
<td>Yes (hospitalization)</td>
</tr>
<tr>
<td>2005</td>
<td>PA</td>
<td>Vaccinia (recombinant WR)</td>
<td>No</td>
<td>Needlestick</td>
<td>Yes</td>
</tr>
<tr>
<td>2005</td>
<td>NM</td>
<td>Vaccinia</td>
<td>No</td>
<td>Eye splash</td>
<td>No</td>
</tr>
<tr>
<td>2005</td>
<td>IA</td>
<td>Vaccinia (recombinant WR)</td>
<td>No</td>
<td>Needlestick</td>
<td>Yes</td>
</tr>
<tr>
<td>2007</td>
<td>NM</td>
<td>Vaccinia</td>
<td>No</td>
<td>Animal care facility</td>
<td>No</td>
</tr>
<tr>
<td>2007</td>
<td>MD</td>
<td>Vaccinia (recombinant WR)</td>
<td>No</td>
<td>Needlestick</td>
<td>No</td>
</tr>
<tr>
<td>2007</td>
<td>NH</td>
<td>Vaccinia (recombinant WR)</td>
<td>No</td>
<td>Needlestick</td>
<td>Yes (hospitalization)</td>
</tr>
<tr>
<td>2007</td>
<td>MA</td>
<td>Vaccinia (recombinant NYCBH)</td>
<td>No</td>
<td>Needlestick</td>
<td>Yes (hospitalization)</td>
</tr>
<tr>
<td>2007</td>
<td>MD</td>
<td>Monkeypox</td>
<td>Yes</td>
<td>Needlestick</td>
<td>No</td>
</tr>
<tr>
<td>2008</td>
<td>CA</td>
<td>Vaccinia (recombinant WR)</td>
<td>No</td>
<td>Eye splash</td>
<td>No</td>
</tr>
<tr>
<td>2008</td>
<td>NH</td>
<td>Vaccinia (recombinant WR)</td>
<td>No</td>
<td>Eye splash</td>
<td>No</td>
</tr>
<tr>
<td>2008</td>
<td>VA</td>
<td>Vaccinia (recombinant WR)</td>
<td>No</td>
<td>Unknown</td>
<td>Yes (hospitalization)</td>
</tr>
<tr>
<td>2008</td>
<td>FL</td>
<td>Vaccinia</td>
<td>Yes</td>
<td>Tube leakage</td>
<td>No</td>
</tr>
</tbody>
</table>

* Detailed account of case provided elsewhere (2008).
5. The possibility exists for inadvertent secondary transmission of VACV from an infected individual. Because of potential delays between laboratory-related exposure and recognition of symptoms, laboratory workers may put contacts, such as family members and healthcare workers, at risk for infection.

Conclusion

The eradication of smallpox through the administration of VACV vaccine is one of the greatest public health achievements in history. VACV remains an important virus today for laboratory-based research, in the study of virology, immunology, and in the development of novel vaccines. However, VACV is potentially pathogenic in humans, and laboratory-acquired infections continue to occur. Although adverse events have been associated with VACV vaccination, post-vaccination symptoms tend to be relatively mild, and the ACIP currently recommends VACV vaccination for laboratory workers who handle non-highly attenuated VACV strains. While the highly attenuated MVA vaccine is not currently licensed in the United States, laboratory workers will hopefully benefit from this vaccine in the future. The usage of proper safety measures, including administration of VACV vaccine, can minimize the risk of conducting research which involves this virus.

Acknowledgments

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References

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